

## EXHIBIT B

**INFORMATION ABOUT PRINCIPAL INVESTIGATORS/PROJECT DIRECTORS(PI/PD) and  
co-PRINCIPAL INVESTIGATORS/co-PROJECT DIRECTORS**

Submit only ONE copy of this form for each PI/PD and co-PI/PD identified on the proposal. The form(s) should be attached to the original proposal as specified in GPG Section II.B. Submission of this information is voluntary and is not a precondition of award. This information will not be disclosed to external peer reviewers. ***DO NOT INCLUDE THIS FORM WITH ANY OF THE OTHER COPIES OF YOUR PROPOSAL AS THIS MAY COMPROMISE THE CONFIDENTIALITY OF THE INFORMATION.***

PI/PD Name: Elliot P Douglas

Gender: ☒ Male ☐ Female

Ethnicity: (Choose one response) ☐ Hispanic or Latino ☒ Not Hispanic or Latino

Race:  
(Select one or more)

☐ American Indian or Alaska Native  
☐ Asian  
☐ Black or African American  
☐ Native Hawaiian or Other Pacific Islander  
☒ White

Disability Status:  
(Select one or more)

☐ Hearing Impairment  
☐ Visual Impairment  
☐ Mobility/Orthopedic Impairment  
☐ Other  
☒ None

Citizenship: (Choose one) ☒ U.S. Citizen ☐ Permanent Resident ☐ Other non-U.S. Citizen

Check here if you do not wish to provide any or all of the above information (excluding PI/PD name): ☒

**REQUIRED:** Check here if you are currently serving (or have previously served) as a PI, co-PI or PD on any federally funded project ☒

**Ethnicity Definition:**

**Hispanic or Latino.** A person of Mexican, Puerto Rican, Cuban, South or Central American, or other Spanish culture or origin, regardless of race.

**Race Definitions:**

**American Indian or Alaska Native.** A person having origins in any of the original peoples of North and South America (including Central America), and who maintains tribal affiliation or community attachment.

**Asian.** A person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam.

**Black or African American.** A person having origins in any of the black racial groups of Africa.

**Native Hawaiian or Other Pacific Islander.** A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.

**White.** A person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

**WHY THIS INFORMATION IS BEING REQUESTED:**

The Federal Government has a continuing commitment to monitor the operation of its review and award processes to identify and address any inequities based on gender, race, ethnicity, or disability of its proposed PIs/PDs. To gather information needed for this important task, the proposer should submit a single copy of this form for each identified PI/PD with each proposal. Submission of the requested information is voluntary and will not affect the organization's eligibility for an award. However, information not submitted will seriously undermine the statistical validity, and therefore the usefulness, of information received from others. Any individual not wishing to submit some or all the information should check the box provided for this purpose. (The exceptions are the PI/PD name and the information about prior Federal support, the last question above.)

Collection of this information is authorized by the NSF Act of 1950, as amended, 42 U.S.C. 1861, et seq. Demographic data allows NSF to gauge whether our programs and other opportunities in science and technology are fairly reaching and benefiting everyone regardless of demographic category; to ensure that those in under-represented groups have the same knowledge of and access to programs and other research and educational opportunities; and to assess involvement of international investigators in work supported by NSF. The information may be disclosed to government contractors, experts, volunteers and researchers to complete assigned work; and to other government agencies in order to coordinate and assess programs. The information may be added to the Reviewer file and used to select potential candidates to serve as peer reviewers or advisory committee members. See Systems of Records, NSF-50, "Principal Investigator/Proposal File and Associated Records", 63 Federal Register 267 (January 5, 1998), and NSF-51, "Reviewer/Proposal File and Associated Records", 63 Federal Register 268 (January 5, 1998).

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**PI/PD Name:** Laurie B Gower

**Gender:** ☐ Male ☒ Female

**Ethnicity:** (Choose one response) ☐ Hispanic or Latino ☒ Not Hispanic or Latino

**Race:**  
(Select one or more) ☐ American Indian or Alaska Native  
☐ Asian  
☐ Black or African American  
☐ Native Hawaiian or Other Pacific Islander  
☒ White

**Disability Status:**  
(Select one or more) ☐ Hearing Impairment  
☐ Visual Impairment  
☐ Mobility/Orthopedic Impairment  
☐ Other  
☒ None

**Citizenship:** (Choose one) ☒ U.S. Citizen ☐ Permanent Resident ☐ Other non-U.S. Citizen

**Check here if you do not wish to provide any or all of the above information (excluding PI/PD name):** ☒

**REQUIRED: Check here if you are currently serving (or have previously served) as a PI, co-PI or PD on any federally funded project** ☒

**Ethnicity Definition:**

**Hispanic or Latino.** A person of Mexican, Puerto Rican, Cuban, South or Central American, or other Spanish culture or origin, regardless of race.

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Collection of this information is authorized by the NSF Act of 1950, as amended, 42 U.S.C. 1861, et seq. Demographic data allows NSF to gauge whether our programs and other opportunities in science and technology are fairly reaching and benefiting everyone regardless of demographic category; to ensure that those in under-represented groups have the same knowledge of and access to programs and other research and educational opportunities; and to assess involvement of international investigators in work supported by NSF. The information may be disclosed to government contractors, experts, volunteers and researchers to complete assigned work; and to other government agencies in order to coordinate and assess programs. The information may be added to the Reviewer file and used to select potential candidates to serve as peer reviewers or advisory committee members. See Systems of Records, NSF-50, "Principal Investigator/Proposal File and Associated Records", 63 Federal Register 267 (January 5, 1998), and NSF-51, "Reviewer/Proposal File and Associated Records", 63 Federal Register 268 (January 5, 1998).

## List of Suggested Reviewers or Reviewers Not To Include (optional)

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### **SUGGESTED REVIEWERS:**

Not Listed

### **REVIEWERS NOT TO INCLUDE:**

Not Listed

# COVER SHEET FOR PROPOSAL TO THE NATIONAL SCIENCE FOUNDATION

PROGRAM ANNOUNCEMENT/SOLICITATION NO./CLOSING DATE// if not in response to a program announcement/solicitation enter NSF 99-2					<b>FOR NSF USE ONLY</b>	
<b>NSF 99-109</b> [REDACTED]					<b>NSF PROPOSAL NUMBER</b>	
FOR CONSIDERATION BY NSF ORGANIZATION UNIT(S) (Indicate the most specific unit known, i.e. program, division, etc.)					<b>9986333</b>	
<b>ENG - Directorate for Engineering</b>						
DATE RECEIVED	NUMBER OF COPIES	DIVISION ASSIGNED	FUND CODE	DUNS# (Data Universal Numbering System)	FILE LOCATION	
[REDACTED]	10			969663814	[REDACTED]	
EMPLOYER IDENTIFICATION NUMBER (EIN) OR TAXPAYER IDENTIFICATION NUMBER (TIN)		SHOW PREVIOUS AWARD NO. IF THIS IS <input type="checkbox"/> A RENEWAL <input type="checkbox"/> AN ACCOMPLISHMENT-BASED RENEWAL		IS THIS PROPOSAL BEING SUBMITTED TO ANOTHER FEDERAL AGENCY? YES <input type="checkbox"/> NO <input checked="" type="checkbox"/> IF YES, LIST ACRONYMS(S)		
596002052						
NAME OF ORGANIZATION TO WHICH AWARD SHOULD BE MADE			ADDRESS OF Awardee ORGANIZATION, INCLUDING 9 DIGIT ZIP CODE			
University of Florida			University of Florida			
AWARDEE ORGANIZATION CODE (IF KNOWN)			1 UNIVERSITY OF FLORIDA			
0015354000			GAINESVILLE, FL. 326112002			
NAME OF PERFORMING ORGANIZATION, IF DIFFERENT FROM ABOVE			ADDRESS OF PERFORMING ORGANIZATION, IF DIFFERENT, INCLUDING 9 DIGIT ZIP CODE			
PERFORMING ORGANIZATION CODE (IF KNOWN)						
IS Awardee ORGANIZATION (Check All That Apply) (See GPG II.D.1 For Definitions) <input type="checkbox"/> FOR-PROFIT ORGANIZATION <input type="checkbox"/> SMALL BUSINESS <input type="checkbox"/> MINORITY BUSINESS <input type="checkbox"/> WOMAN-OWNED BUSINESS						
TITLE OF PROPOSED PROJECT <b>Nanostructured Composites via Biomimetic Processing</b>						
REQUESTED AMOUNT \$ [REDACTED]		PROPOSED DURATION (1-60 MONTHS) [REDACTED]		REQUESTED STARTING DATE [REDACTED]		SHOW RELATED PREPROPOSAL NO., IF APPLICABLE
CHECK APPROPRIATE BOX(ES) IF THIS PROPOSAL INCLUDES ANY OF THE ITEMS LISTED BELOW						
<input type="checkbox"/> BEGINNING INVESTIGATOR (GPG 1.A.3)			<input type="checkbox"/> VERTEBRATE ANIMALS (GPG II.D.12) IACUC App. Date _____			
<input type="checkbox"/> DISCLOSURE OF LOBBYING ACTIVITIES (GPG II.D.1)			<input type="checkbox"/> HUMAN SUBJECTS (GPG II.D.12)			
<input type="checkbox"/> PROPRIETARY & PRIVILEGED INFORMATION (GPG II.D.10)			Exemption Subsection _____ or IRB App. Date _____			
<input type="checkbox"/> NATIONAL ENVIRONMENTAL POLICY ACT (GPG II.D.10)			<input type="checkbox"/> INTERNATIONAL COOPERATIVE ACTIVITIES: COUNTRY/COUNTRIES			
<input type="checkbox"/> HISTORIC PLACES (GPG II.D.10)			_____			
<input type="checkbox"/> SMALL GRANT FOR EXPLOR. RESEARCH (SGER) (GPG II.D.12)			<input type="checkbox"/> FACILITATION FOR SCIENTISTS/ENGINEERS WITH DISABILITIES (GPG V.G.)			
<input type="checkbox"/> GROUP PROPOSAL (GPG II.D.12)			<input type="checkbox"/> RESEARCH OPPORTUNITY AWARD (GPG V.H)			
PI/PD DEPARTMENT <b>Materials Science &amp; Engineering</b>		PI/PD POSTAL ADDRESS <b>323 MAE Building</b>				
PI/PD FAX NUMBER <b>352-392-3771</b>		<b>PO Box 116400</b>				
		<b>Gainesville, FL 326116400</b>				
		<b>United States</b>				
NAMES (TYPED)	High Degree	Yr of Degree	Telephone Number	Electronic Mail Address		
PI/PD NAME <b>Elliot P Douglas</b>	PhD	1993	352-846-2836	edoug@mse.ufl.edu		
CO-PI/PD <b>Laurie B Gower</b>	PhD	1997	352-846-3336	lgowe@mse.ufl.edu		
CO-PI/PD						
CO-PI/PD						
CO-PI/PD						

## CERTIFICATION PAGE

### Certification for Principal Investigators and Co-Principal Investigators:

I certify to the best of my knowledge that:

- (1) the statements herein (excluding scientific hypotheses and scientific opinions) are true and complete, and  
 (2) the text and graphics herein as well as any accompanying publications or other documents, unless otherwise indicated, are the original work of the signatories or individuals working under their supervision. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if an award is made as a result of this application.

I understand that the willful provision of false information or concealing a material fact in this proposal or any other communication submitted to NSF is a criminal offense (U.S. Code, Title 18, Section 1001).

Name (Typed)	Signature	Social Security No.*	Date
PI/PD <b>Elliot P Douglas</b>		*ON FAST LANE SUBMISSIONS* SSNs are confidential and are not displayed	
Co-PI/PD <b>Laurie B Gower</b>			
Co-PI/PD			
Co-PI/PD			
Co-PI/PD			

### Certification for Authorized Organizational Representative or Individual Applicant:

By signing and submitting this proposal, the individual applicant or the authorized official of the applicant institution is: (1) certifying that statements made herein are true and complete to the best of his/her knowledge; and (2) agreeing to accept the obligation to comply with NSF award terms and conditions if an award is made as a result of this application. Further, the applicant is hereby providing certifications regarding Federal debt status, debarment and suspension, drug-free workplace, and lobbying activities (see below), as set forth in Grant Proposal Guide (GPG), NSF 99-2. Willful provision of false information in this application and its supporting documents or in reports required under an ensuing award is a criminal offense (U. S. Code, Title 18, Section 1001).

In addition, if the applicant institution employs more than fifty persons, the authorized official of the applicant institution is certifying that the institution has implemented a written and enforced conflict of interest policy that is consistent with the provisions of Grant Policy Manual Section 510; that to the best of his/her knowledge, all financial disclosures required by that conflict of interest policy have been made; and that all identified conflicts of interest will have been satisfactorily managed, reduced or eliminated prior to the institution's expenditure of any funds under the award, in accordance with the institution's conflict of interest policy. Conflict which cannot be satisfactorily managed, reduced or eliminated must be disclosed to NSF.

#### Debt and Debarment Certifications

(If answer "yes" to either, please provide explanation.)

Is the organization delinquent on any Federal debt?

Yes ☐

No ☒

Is the organization or its principals presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department or agency?

Yes ☐

No ☒

#### Certification Regarding Lobbying

This certification is required for an award of a Federal contract, grant, or cooperative agreement exceeding \$100,000 and for an award of a Federal loan or a commitment providing for the United States to insure or guarantee a loan exceeding \$150,000.

#### Certification for Contracts, Grants, Loans and Cooperative Agreements

The undersigned certifies, to the best of his or her knowledge and belief, that:

(1) No federal appropriated funds have been paid or will be paid, by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan, or cooperative agreement.

(2) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with this Federal contract, grant, loan, or cooperative agreement, the undersigned shall complete and submit Standard Form-LLL, "Disclosure Form to Report Lobbying," in accordance with its instructions.

(3) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers including subcontracts, subgrants, and contracts under grants, loans, and cooperative agreements and that all subrecipients shall certify and disclose accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction was made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by section 1352, title 31, U.S. Code. Any person who fails to file the required certification shall be subject to a civil penalty of not less than \$10,000 and not more than \$100,000 for each such failure.

AUTHORIZED ORGANIZATIONAL REPRESENTATIVE		SIGNATURE	DATE
NAME/TITLE (TYPED) <b>Sandra Goldstein</b>			<b>08/12/99</b>
TELEPHONE NUMBER <b>352-392-1582</b>	ELECTRONIC MAIL ADDRESS <b>sandyg@ufl.edu</b>	FAX NUMBER <b>352-392-9605</b>	

\*SUBMISSION OF SOCIAL SECURITY NUMBERS IS VOLUNTARY AND WILL NOT AFFECT THE ORGANIZATION'S ELIGIBILITY FOR AN AWARD. HOWEVER, THEY ARE AN INTEGRAL PART OF THE INFORMATION SYSTEM AND ASSIST IN PROCESSING THE PROPOSAL. SSN SOLICITED UNDER NSF ACT OF 1950, AS AMENDED.

## A. Project Summary

We propose to create novel nanostructured ceramic composites which mimic the structure of pseudolamellar (parallel-fibered) bone. These composites will have enhanced toughness and high strength due to the arrangement of nanocrystalline ceramic particles within an ordered collagen matrix. Such materials will have specific applications as bone-graft substitutes for repair of critical size osseous defects. In addition, the regulation of properties through control of nanostructure will demonstrate a general strategy for the development of polymer-ceramic composites for structural applications.

The creation of these composites relies on novel processing techniques currently under active investigation by the two investigators of this proposal. Magnetic field processing of collagen will be used to control the orientation of the organic framework. Subsequent mineralization of the collagen will be accomplished via the Polymer Induced Liquid Precursor (PILP) process. The PILP process utilizes a metastable, highly supersaturated liquid-phase precursor to the inorganic phase, which is stabilized by an ionic polymer that is a simple mimic to the acidic proteins associated with biominerals. Because the mineral precursor is in the form of a liquid phase, it is proposed to utilize the capillary action of collagen fibrils to draw the precursor up into the grooves of the collagen. Subsequent solidification and crystallization of the precursor within the collagen fibrils will create an oriented, nanocrystalline structure. We suggest that this may be the mechanism used by organisms to create the unique intrafibrillar mineralization of collagen during bone formation. Thus, through regulation of the orientation and architecture of the collagenous framework, which in turn controls the orientation of the nanocrystals aligned within the collagen fibrils, the mechanical properties can be modulated. In order to accomplish these goals, the specific tasks of the project are as follows:

- [REDACTED]
- PILP Mineralization: A liquid-phase precursor will be generated for calcium phosphate using the highly acidic proteins associated with biominerals or their synthetic analogues. The liquid precursor will be incorporated into partially dried collagen gels via capillary action, followed by intrafibrillar mineralization of the organized gels. Optimization of the mineralization process will be accomplished by varying the ionic polymer, the degree to which the gels are dried, and the crystallization conditions. The nanostructure of the composites will be examined via x-ray diffraction, electron diffraction, scanning electron microscopy, and transmission electron microscopy.
- Mechanical Properties: Flexural properties of the composites will be measured using a standard 4-point bend test. In addition, the fracture toughness will be determined using fractography.

Overall, the broad goal of this research is to provide a new route to ceramic composites with controlled structure and properties. Nature has already learned how to combine high strength and high fracture toughness through careful control of the architecture of bone. In particular, the spatial constraints created by intrafibrillar mineralization of collagen dictate the size of the nanocrystallites, as well as the anisotropic shape and orientation. This structure provides a unique reinforcing phase that generates non-interacting microcracks for enhanced toughness of the bioceramic. By mimicking this architecture with the advanced processing techniques described above, it will be possible to create a new class of structural ceramics.

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For font size and page formatting specifications, see GPG section II.C.

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Cover Sheet (NSF Form 1207) (Submit Page 2 with original proposal only)		
A Project Summary (not to exceed 1 page)	1	_____
B Table of Contents (NSF Form 1359)	1	_____
C Project Description (plus Results from Prior NSF Support) (not to exceed 15 pages) <b>(Exceed only if allowed by a specific program announcement/solicitation or if approved in advance by the appropriate NSF Assistant Director or designee)</b>	15	_____
D References Cited	4	_____
E Biographical Sketches (Not to exceed 2 pages each)	4	_____
F Budget (NSF Form 1030, plus up to 3 pages of budget justification)	4	_____
G Current and Pending Support (NSF Form 1239)	4	_____
H Facilities, Equipment and Other Resources (NSF Form 1363)	1	_____
I Special Information/Supplementary Documentation	0	_____
J Appendix (List below. ) <b>(Include only if allowed by a specific program announcement/ solicitation or if approved in advance by the appropriate NSF Assistant Director or designee)</b>	_____	_____
Appendix Items:		

\*Proposers may select any numbering mechanism for the proposal. The entire proposal however, must be paginated. Complete both columns only if the proposal is numbered consecutively.

NSF Form 1359 (10/99)

## C. PROJECT DESCRIPTION

### Results from Prior NSF Support

The PI (Dr. Elliot Douglas) has previously received a grant from the NSF Division of Undergraduate Education. This is an education related project, and has no relation to the current proposal. The co-PI (Dr. Laurie Gower) has not previously received NSF funding as PI or co-PI. However, a portion of her start-up funds was provided by the NSF Engineering Research Center for Particle Science & Technology.

### 1. Research Objectives and Significance

The objective of the proposed research is as follows:

*To combine magnetic field processing of collagen with polymer-induced liquid precursor (PILP) mineralization to create a biomimetic material with nanostructured architecture and mechanical properties that mimic those of natural bone.*

This project presents a novel approach to creating biomimetic structures. Particularly, it relies on the expertise of the two investigators of this project. Dr. Laurie Gower recently discovered the PILP process. Based on the unique attributes of this process, and correspondingly similar features of biominerals, Gower has proposed that a similar precursor processing mechanism may be utilized by organisms to regulate crystal morphologies in biominerals. Therefore, it is anticipated that this novel mineralization technique will provide the possibility to create, for the first time, a synthetic material with the specific uniformly-aligned nanocrystalline structure of natural bone.

The material to be developed under this proposal has specific applications in biomedical engineering. We have proposed that this material may be useful as a bone graft for repair of critical size osseous defects. The advantages of such a material are primarily that it will be resorbable, and thus remodeled into natural bone as the defect heals, and that the biomimetic structure will exhibit mechanical properties similar to that of natural bone. The first point is addressed in other proposals by the investigators. This proposal addresses the issue of mechanical properties, with focus on the relationship of the nanostructured architecture of the mineralized composites.

In addition to specific biomedical engineering applications, the composite material developed will have applications in other structural areas. Use of ceramics in structural applications is often limited by their inherent brittleness. A number of advances have been made, but it is still a challenge to create a material that retains the high strength of ceramics with enhanced fracture toughness. Nature has overcome this problem in bone by combining an organic framework (collagen) with a nanocrystalline ceramic (hydroxyapatite) to create a strong, yet tough composite. Through regulation of the orientation and architecture of the collagenous framework, which in turn controls the orientation of the nanocrystals aligned within the collagen fibrils, the mechanical properties can be modulated. By mimicking this structure through the novel

processing techniques described above, we will be able to create a new class of structural ceramic materials which derive enhanced fracture toughness through the nanostructuring of the ceramic phase.

## 2. Background

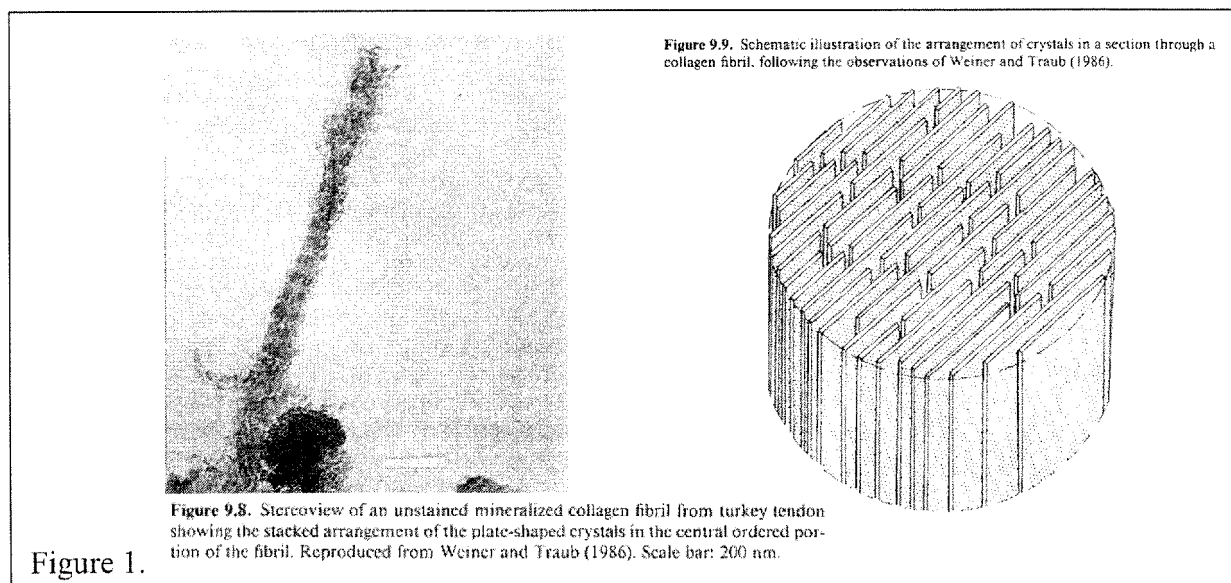
### 2.1. Structure and Properties of Bone

Our approach to the fabrication of bone-graft substitutes is novel in that we will use a biomimetic processing technique that has the potential to deliver a mineral-collagen composite with an architecture that mimics the ultrastructure of parallel-fibered bone. Carter defines parallel-fibered bone as consisting of a large quantity of closely-packed collagen fibrils with the same general orientation, that is, running approximately parallel to each other.<sup>1</sup> This type of microstructure is found in the lamellar-zonal region of primary bone tissue, and in the bone trabeculae lining the medullary sinuses. In the primary osteons of bony fishes and birds, the fibrous matrix may also be parallel-fibered, as well as in ossified tendons of birds.

An in depth description of the biomimetic processing technique, called the Polymer-Induced Liquid-Precursor (PILP) process, is provided below. In brief, an acidic polymeric additive stabilizes an amorphous precursor to the mineral that is in the form of a liquid. Upon solidification of the precursor, non-equilibrium morphologies are generated that are very different than those that occur in a typical solution crystallization process. In particular, we propose that the fluid consistency of the precursor could be drawn up into the collagen fibrils via capillary action, which would fill the grooves of the collagen fibrils with a highly supersaturated, metastable phase. Upon solidification of the precursor, a favorably mineralized, nanostructured composite would be generated, which we predict will mimic the nanostructure of bone. Upon solidification of the precursor, the dimensions of the hydroxyapatite (HAP) crystallites will be restrained by the surrounding collagen fibrils, and if the solidification process transforms through a singular nucleation event, the crystallites should exhibit uniform orientation, as is the case for mineralized collagen in bone.

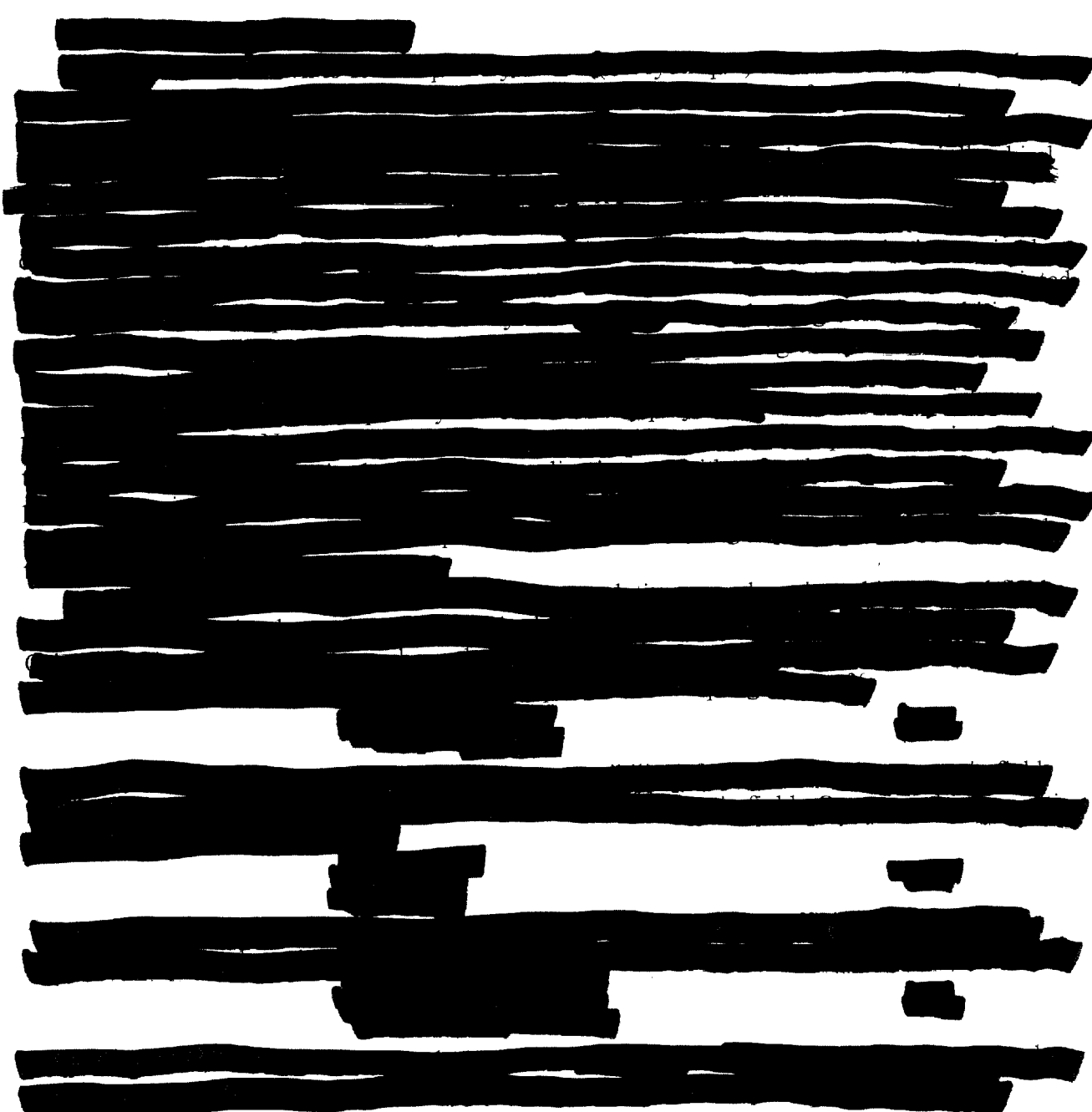
Studies on the mineralization of turkey tendon have provided important information regarding the location of HAP crystallites in the collagen fibrils of bone. Figure 1 shows illustrations taken from the book On Biomineralization<sup>2</sup> of a mineralized collagen fibril from turkey tendon, and a schematic of the classic “deck-of-cards” arrangement of the nanocrystals within the fibril. Throughout this discussion, we will refer to this type of mineralization of collagen as *intrafibrillar*, to distinguish it from the typical *interfibrillar* mineralization that is produced *in vitro*. In the latter case, the hydroxyapatite crystallizes on and around the collagen fibrils, but does not fill the gaps and grooves to form an intrafibrillar composite.<sup>3-8</sup> This leads to much lower loading of mineral phase, and thus the mechanical properties are not similar to those of bone/dentin. It is desirable to fabricate bone implant materials that have a modulus that matches that of the surrounding hard tissue so that stress shielding does not lead to excessive resorption of surrounding bone tissue.<sup>9-12</sup> It is the intrafibrillar mineralization that we seek to duplicate because we believe this nanostructural level of organization is a primary determinant of the unique mechanical properties of bone.

The ultrastructure of bone tissue can be considered to be a hierarchy of structural control. For example, the alignment of HAP nanocrystals within the fibrils provides a level of nanostructural control, which is then organized at the microstructural level through organization of the collagenous phase. We have focused on the parallel-fibered matrix because it is the most readily



attainable using current synthetic techniques. Parallel-fibered bone can be considered intermediate between woven bone, which consists of a random organization of collagen fibrils, and lamellar bone, which consists of individual lamellae containing parallel fibrils, but which are spatially organized into an alternating plywood-like structure.<sup>1</sup> Parallel-fibered bone is sometimes referred to as pseudolamellar. The parallel arrangement of fibrils leads to a parallel arrangement of the aligned nanocrystals within the fibrils, and thus leads to considerable anisotropy of properties of the composite (both optical and mechanical),<sup>13</sup> such as for example the relatively large compressive modulus and strength of bone. As Calvert notes,<sup>14</sup> not only is the arrangement of the matrix enviable to the materials engineer, but the high-loading density of the mineral phase is another desirable feature that is difficult to achieve synthetically. The high degree of mineralization imparts a high compressive modulus (relative to soft tissues), which can be directionally regulated by alignment of the collagen in accordance with the stress fields imposed on the bone tissue, according to Wolff's Law. Another appealing aspect of the nanostructural arrangement of bone tissue is that it likely plays a role in imparting toughness to the bioceramic material. The disjointed arrangement of the brittle phase (mineral crystals) within the ductile matrix (collagen fibrils) presumably disrupts the propagation of cracks and absorbs the fracture energy, thus providing a unique combination of strength and toughness, that along with the stiffness, provides the optimal material for a skeletal support system. To our knowledge, a bioresorbable material that mimics the mechanical properties of bone has not been fabricated to date. Such a load-bearing biomaterial would be desirable because it could diminish the stress shielding effect that occurs from implants that are stiffer than the surrounding bone. Even more problematic is how to produce a biomaterial with matching mechanical properties that are maintained at a sufficient level as the implant material is degraded and replaced by natural bone tissue. To accomplish this, we believe that it is imperative that the implant be bioresorbable, and not just biodegradable, because the rates of bone ingrowth will never be consistent among different individuals, or even in different locations within one individual. The remodeling process needs to be under cellular control to provide the appropriate maintenance of mechanical properties during the remodeling, as occurs in natural bone regeneration. With this goal in mind, we consider the biomimetic approach to be a prerequisite for achieving the most favorable synthetic bone-graft substitute, that is, one that faithfully mimics the ultrastructure of bone.

We note that the parallel-fibered architecture is not optimal for torsional properties. Biologically, the remodeling of primary bone into secondary osteons provides a means for enhancing the torsional properties. An osteonic type of architecture is beyond the scope of this initial project. However, we note that a splay in the liquid-crystalline orientation may occur upon release of the magnetic field, and this may actually enhance the torsional properties of our biomaterial. The natural tendency for collagen to assemble into a cholesteric liquid-crystal phase has been proposed to be a mechanism that is utilized for the changing orientation of the fibrils within lamellae.<sup>15</sup> Thus, a perfect parallel-fibered arrangement may not be the most desirable microstructure, and as such, this issue does fall within the scope of the project and will be investigated with respect to mechanical properties.



[REDACTED]

[REDACTED]

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### ***2.3. PILP Mineralization***

As discover of the PILP process, Dr. Gower is uniquely qualified for the proposed studies. To date, the PILP process has been demonstrated for calcium carbonates.<sup>32-34</sup> Calcium phosphates (CaP) and oxalates are currently being investigated to determine a means of inducing

a liquid precursor in these mineral systems (preliminary results for CaP are discussed below). The overall description of the PILP process can be summarized as follows: An acidic macromolecular additive transforms the solution crystallization of an ionic crystal to a solidification process of an amorphous precursor. Although it is no surprise that a metastable amorphous phase will transform to the more stable crystalline state, the novelty of the PILP process is that the polymer additive stabilizes a metastable liquid-phase precursor to the mineral. The metastable phase is a distinct entity, in which boundaries of isolated liquid droplets are observed in the aqueous solution. The droplets presumably contain polymeric additive (compositions currently under investigation), but this phase does not behave like a gel, and the final mineral product subsequent to solidification has undetectable amounts of polymer within the mineral (as determined by energy dispersive spectroscopic analysis of PILP deposited calcitic films). More importantly, the mineral crystals assume the shape of the amorphous precursor, rather than forming the faceted habits that are typical of solution grown crystals. Because the precursor is initially a liquid, it can take on a variety of shapes, and it retains this shape even though the transformed crystal exposes energetically unfavorable crystal surfaces (including curved surfaces on single crystals). For example, droplets in solution crystallize to form single crystal “drops”, or the droplets deposit on a substrate and coalesce into tablets, films, and coatings, or the droplets can fill the space of a compartment to form “spatially-delineated” single crystals. Notably, such non-equilibrium morphologies are commonly observed in calcium carbonate biominerals, and in particular, the calcium carbonate tablets that comprise the nacre of mollusk shells have strikingly similar morphologies and distinctive defect textures that match the in vitro PILP deposited tablets.<sup>35-39</sup>

The chemical mechanism underlying the PILP process is still under investigation, but it likely involves the sequestering ability of polyanionic species that have an affinity for calcium ions. In our reports, we have suggested that the PILP process may be related to the “ionotropic nucleation” mechanism described by Greenfield and Crenshaw,<sup>40, 41</sup> in which the initial ion binding attracts the counterion species, which then draws in more cations until the region becomes increasingly supersaturated with the ions. Presumably the polymer inhibits the nucleation (or growth) long enough to stabilize the highly supersaturated region, which then phase separates from the aqueous solution as some critical density is achieved. Interestingly, the formation of metastable phases, including liquid ones, was documented long ago by Ostwald, but is usually lost in translation. In the classic book On Growth and Form,<sup>42</sup> D’Arcy Thompson discusses the formation of rounded concretions, and says the following:

*“In accordance with a rule first recognized by Ostwald, when a substance begins to separate from a solution, so making its first appearance as a new phase, it always makes its appearance first as a liquid.”*

#### Is the PILP process relevant to calcium phosphate biomineralization?

Calcium carbonates and calcium phosphates exhibit very different crystal morphologies, both synthetically and in biominerals.<sup>2</sup> For example, calcium carbonate biominerals commonly form relatively large crystals with shapes that appear to have been “molded” by the compartment within which they form; whereas the calcium phosphate biominerals form very small crystals with fairly normal crystal habits (albeit quite elongated in enamel rods).<sup>2, 43, 44</sup> In the calcium phosphate biominerals, it is the arrangement of the crystallites that is more puzzling, rather than the shapes. After reviewing the literature on osteogenesis,<sup>9, 10, 36, 45, 46</sup> dentinogenesis,<sup>45</sup> and amelogenesis,<sup>2, 44</sup> several concepts strike the investigator as being potentially relevant to a liquid-precursor process.

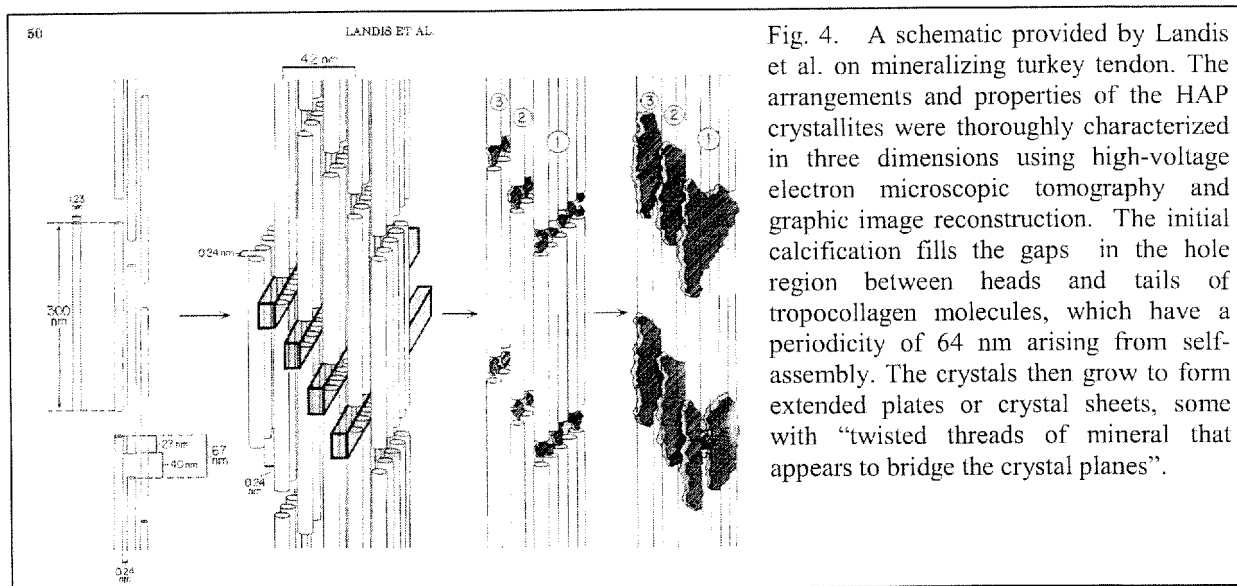


Fig. 4. A schematic provided by Landis et al. on mineralizing turkey tendon. The arrangements and properties of the HAP crystallites were thoroughly characterized in three dimensions using high-voltage electron microscopic tomography and graphic image reconstruction. The initial calcification fills the gaps in the hole region between heads and tails of tropocollagen molecules, which have a periodicity of 64 nm arising from self-assembly. The crystals then grow to form extended plates or crystal sheets, some with "twisted threads of mineral that appears to bridge the crystal planes".

The most supportive argument is based on the unusual characteristics of the HAP crystallites deposited within collagen fibrils. Many investigators have contributed to these types of studies,<sup>8, 47-50</sup> but the work of Landis et al.<sup>51, 52</sup> on mineralizing turkey tendon is particularly appropriate to this discussion. The issue of crystal size and shape has been somewhat controversial, but their observations (for this particular collagen), show the crystals to be platelet-shaped, but with irregular edges and variable lengths (see Fig. 4). The crystals grow to form extended plates or crystal sheets, some with "twisted threads of mineral that appears to bridge the crystal planes". They also describe an apparent curving of the mineral where crystals may be located on the surface of a collagen fibril. The enlargement of crystal widths seems to "imply that mineral is accommodated by channels of grooves in collagen formed by adjacent hole zones in register," correlating to the quarter-staggered model of collagen assembly first proposed by Hodge and Petraska.<sup>47</sup> Interestingly, the crystals in localized regions appear to form at different sites and times by independent nucleation sites, yet they grow to interconnect and fuse into larger coplanar units from the smaller individual particles. Lastly, they observe a thin envelope of organic origin that is present in a location that defines the location of the crystallites (determined by density). We note that a liquid-phase precursor could provide a viable explanation for some of the unusual features they describe, such as curving and fusion of crystals, and lack of singular location of nucleation. These are all features that have been observed in the PILP process for calcium carbonates.<sup>32-34</sup> The envelope surrounding the crystals could be excluded molecules that had stabilized the precursor phase. We are examining the calcite system to determine if the polymer gets excluded during solidification, or if it remains occluded within the crystals. However, at this nanoscopic size scale of CaP biomineralization, it seems more likely that large macromolecules would be excluded as the precursor phase crystallizes.

In conclusion, the literature commonly alludes to the belief that there is some unifying mechanism among differing biomineral systems. We suggest that the PILP process, in conjunction with well established mechanisms of biomineralization, can provide a viable explanation for many of the puzzling morphologies of biominerals, including those of the calcium phosphate biominerals in bones and teeth. Some examples include the following: the "inorganic substance in bands" (ISB) described by Bonucci (PILP dehydration to amorphous granular precursor);<sup>45</sup> the elongation of enamel prisms generated by appositional growth during

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A large, stylized black silhouette of a traditional Chinese gate or structure. It features a horizontal beam supported by four vertical posts, with a thick base and a decorative top. The silhouette is set against a white background.

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### **3.2. PILP Mineralization**

#### Experimental Overview

- ◆ Determine optimal reaction conditions for generating a PILP phase in calcium phosphates
  - test crystallization conditions relevant to physiological environment
  - test a variety of synthetic acidic macromolecular additives
  - test the highly acidic macromolecules extracted from bone/dentin (e.g. phosphophoryn, osteopontin, bone sialoprotein, etc.)

- ◆ Mineralize collagen fibrils via capillary action by placing ends into a PILP media
  - run a suitable control reaction (collagen placed in supersaturated crystallizing solution)
  - determine the influence of collagen variables on the mineralization process (densification, orientation)
- ◆ Characterize mineralized collagen fibrils and compare/contrast with fibrils from bone
  - determine composition of collagenous composites for relative content of inorganic phase
  - determine crystallite size, phase and orientation by SEM, TEM, and diffraction:
    - XRD for bulk material
    - ED for individual crystallites in isolated fibrils
  - determine crystal location in isolated fibrils by TEM analysis
  - Study the alignment of HAP crystallites within collagen that displays an iso-oriented mosaic texture, using high-resolution synchrotron XRD

## Materials and Methods

### **a. Eliciting the PILP Process in Calcium Phosphates**

We are currently using a synthesis developed by LeGeros, for a “direct fast method” of synthesis of octacalcium phosphate (OCP), and which can also be modified to produce HAP.<sup>43</sup> This synthesis is a stepwise addition of a 0.04M Ca(Ac)<sub>2</sub> solution into a 0.04M NaH<sub>2</sub>PO<sub>4</sub> solution over 1 hour, held at a temperature of 70°C and initially adjusted to pH 4. To generate a PILP phase, small quantities of acidic polymer are added to the crystallizing solution. For example, in the calcium carbonate (CaCO<sub>3</sub>) system, 20 µg/ml of polyaspartic acid is used to elicit a PILP phase. We have initiated feasibility studies for PILP formation in calcium phosphates using the same polymer. The preliminary studies look promising; some features of the crystal products resemble those that form in PILP deposited CaCO<sub>3</sub>. Smooth, concentrically-layered “soft” spheroids that are partially birefringent are initially formed in the solutions. They appear to be hollow spheres, rather than solid spherulitic structures. The control reaction (without polymeric additive) leads to clusters of needle-shaped crystals, presumably OCP, along with a few “hard” spherical particles. In some cases, “globs” of spheres are connected that appear to have coalesced, unlike the solid spheres of the control reaction. Interestingly, the spheroids generated by the polymeric additive appear to “flow” onto the substrate to form a “film”, while the control reaction spheres do not, and are evidently more solidified. We conclude that the polymer-stabilized spheroids have a somewhat fluid or gelatinous consistency as they deposit onto the substrate. More work needs to be done to catch the transformation of these particles in an earlier stage in order to definitively conclude that they initially formed as a liquid precursor, and especially as a state that is fluid enough to be drawn up by capillary action.

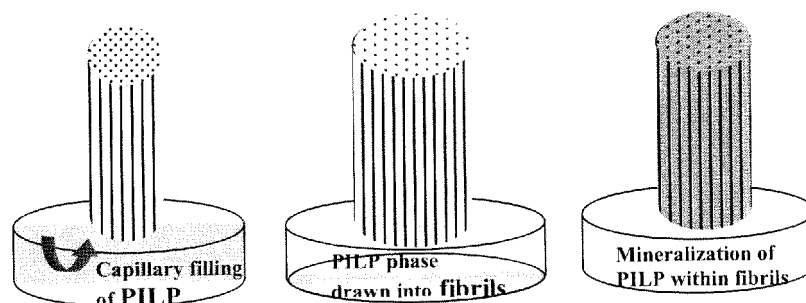
We plan to test other acidic macromolecules as well, and in particular, the highly phosphorylated ones that are commonly found associated with calcium phosphate biominerals (e.g. osteopontin, phosphophoryn),<sup>45, 57</sup> These may be expected to have a stronger affinity for the ions and a more pronounced effect on crystal growth in the calcium phosphate system. For example, Bovine Phosphophoryn is the most acidic protein that has been identified, containing 457 residues of Ser/Pser and 401 residues Asp, per 1000 amino acid residues.<sup>36, 43</sup> Bovine teeth will be decalcified and the insoluble versus soluble fractions separated. The soluble extracts will be characterized by SDS-PAGE (with staining for acidic proteins), purified by the CaCl<sub>2</sub> precipitation procedure using DEAE-HPLC (Rahima and Vies 1988), along with amino acid analysis of particular samples of interest. Other acidic macromolecules of interest include the

Gla-proteins ( $\gamma$ -carboxyglutamic acid containing proteins) of bone and dentin; the acidic glycoproteins extracted from bone, of which there are a variety, such as osteopontin, sialoproteins, etc.; the acidic glycoproteins in  $\text{CaCO}_3$  biominerals, such as the phosphorylated proteins of oyster shells;<sup>36, 58-60</sup> and finally mucopolysaccharides. To further our understanding of the process, simple phosphorylated polypeptides will be synthesized as model compounds to study the physicochemical aspects of the PILP process, such as the effects of varying degree of phosphorylation on the ability to induce the PILP process, and the corresponding characteristics of the PILP phase, etc..

If we are unable to generate a PILP phase for the calcium phosphates, there is the option of using the calcium carbonate system, for which the PILP process was discovered. Calcium carbonates are biocompatible, and it has been shown that coral can be used as a successful bone-graft substitute, and surprisingly, had better osteoinductive potential than did the coral converted to calcium phosphate.<sup>61</sup> It would be of interest to determine if nanocrystals of calcite can also be generated by constraints from the collagen framework. The calcium carbonate crystals in most biomineral examples tend to be larger, such as mollusk shells and sea urchin spines, etc., so it is not clear if there is a physical reason for the use of calcium phosphates by the vertebrates, or if it is more related to metabolic function. In either case, this technique is expected to lead to a structured composite with unique and tailorable mechanical properties.

### b. Synthesis of Mineralized Collagen Composites

Disks of oriented and densified collagen will be prepared such that it is sliced perpendicular to the long axis of the collagen fibrils to expose more ends for facilitating capillary action. The collagen disk will be placed in a PILP media, either as the original aqueous biphasic solution, or isolated and collected as a singular PILP fluid. A control reaction will be run simultaneously, which contains only pure calcifying solution; for example, 2.4mM  $\text{Ca}^{2+}$ , 1.44 mM  $\text{PO}_4^{3-}$ , buffered to pH 7.4 with 40mM HEPES.<sup>6</sup> After sufficient reaction time (to be determined), the collagen will be removed, rinsed, and freeze-dried for characterization.



If the capillary action of the partially dehydrated fibrils proves insufficient for drawing the PILP phase into the fibrils, another alternative approach is to carry out the fibrillogenesis within the liquid-precursor phase. In fact, the unique characteristics of the PILP phase may play a role in the self-assembly of collagen fibrils, thus this would be an interesting possibility to explore. For example, the PILP phase has a high concentration of ions, which could alter the surface energetics between protein molecules, such as driving the assembly of fibrils via hydrophobic interactions, or altering the bridging and crosslinks between fibrils. It is possible that this occurs biologically (although we speculate that the matrix vesicles play a role in delivering precursor phase to fibrils, or at least in the formation of primary bone).

### c. Characterization

As indicated in the overview of methods, the collagenous composites will be characterized for inorganic content. Calcium content can be determined by atomic absorption spectrophotometry of ashed tissue placed in 10% lanthanum in 50% HCl, and phosphorous content as molybdovanadophosphoric acid.<sup>6</sup> The crystal phase of the calcium phosphate will be determined by XRD on deproteinized material (and ED or FTIR if necessary). Crystal size can be determined from XRD peak widths for very thin crystals, which can then be correlated to direct observations by TEM of crystallites extracted from isolated fibrils. XRD will also be used to examine crystal orientation in relation to the collagen fibrils within the disk. For more precise characterization of crystal arrangement, synchrotron XRD will be used to study the mosaic spread of the “single-crystalline” fibers, or iso-oriented matrix of crystallites.

### 3.3. Mechanical Properties

The primary focus of this proposal is to demonstrate the feasibility of using the processing techniques described above to create biomimetic structures with mechanical properties similar to those of natural bone. On average, ordinary bone contains about 60 wt% mineral, 28 wt% protein (mainly collagen), and 12 wt% water.<sup>62, 63</sup> The volume fractions of mineral and organic constituents are roughly equal at 40 vol%, thus it is not entirely obvious which constituents should be considered the matrix phase and which the reinforcing phase. Currey describes bone as being a composite material whereby a tough collagen matrix is reinforced with hydroxyapatite (HAP) platelets.<sup>62</sup> Additionally, the water content plays a significant role in controlling the mechanical properties. The high toughness of bioceramics is thought to result from the presence of organic layers surrounding the crystallites, as well as their highly anisotropic shape.<sup>63</sup> For example, the high axial ratio of the HAP platelets (4nm x 30-50nm x 100-500nm) should provide good load transfer from the matrix to the platelets if the interfacial bonding is strong (relatively little is known about the interfacial bonding in bioceramics). In synthetic fiber composites, the general requirement is to have the filler-matrix interface be weak enough to deflect fracture, yet strong enough to transfer load. Calvert suggests that these two functions may be separated in bone, which has a very fine and well bonded filler, which is combined with weak planes every few microns.<sup>63</sup>

The mechanical properties of bone have been notoriously difficult to analyze because bone can have different microstructures (e.g. fibrous versus lamellar), and has many levels of organization (e.g. osteons of Haversian system).<sup>64</sup> Therefore, a wide range of mechanical properties have been reported. Currey,<sup>62</sup> who has examined the mechanical properties of a variety of biomineral tissues, reports the tensile strength for bone ranges from 20 to 260 MPa, Young's modulus from 5 to 35 GPa, and work of fracture from 20 to 7 kJm<sup>-2</sup>. Other reports for cortical bone have measured an elastic modulus of 20 GPa, tensile strength of 220 MPa, (bend strength of 5 to 150 MPa), strain to break of 10%, and fracture toughness of 5 MPa m<sup>-1/2</sup>.<sup>63, 65</sup>

In order to test the mechanical properties of the biomimetic composites, 4-point flexural measurements based on ASTM C1341 will be utilized. The specimen geometry is 2 mm x 6 mm x 45 mm, with the specimens cut from 2 mm thick plates using a diamond saw. Flexural modulus, strength, strain at break, and work of fracture will be determined from an average of at least 10 samples using the procedures described in ASTM C1341. These properties will be measured as a function of the collagen processing conditions described in section 3.1. Therefore, it will be possible to create an empirical model describing the variation in properties based on the statistical design using the properties as the response variable. In addition, comparison of

changes in properties with the change in birefringence of the collagen gels and the nanostructural details of the ceramic/collagen composite will allow us to specifically identify how these morphological features may be used to control the properties of these materials. For example, the ability to orient the collagen fibrils and their associated crystallites within the fibrils will be expected to lead to highly anisotropic properties, which can be mediated through orientation of the collagenous framework. Likewise, the mineral volume fraction will be measured, which is expected to play a key role in regulation of modulus and strength, as for the case of bone (but with a concomitant loss in toughness).

One of the advantages of the biomimetic structure described earlier is the possibility of combining high strength with high fracture toughness. The disjointed arrangement of the brittle phase (mineral crystals) within the ductile matrix (collagen fibrils) is expected to disrupt the propagation of cracks and absorb the fracture energy, thus providing a unique combination of strength and toughness. The high work to fracture that has been measured for bone and other bioceramics is believed to result from extensive microcracking (e.g.  $W_f = 1710 \text{ J/m}^2$  for bovine femur).<sup>62</sup> The microcracks are generally non-interacting, allowing stress concentrators to be blunted and considerable microdamage to accumulate without catastrophic failure.<sup>66</sup> The load-deformation curves (tensile) for compact bone show residual strain and compliance during successive loading, which presumably results from microdamage accumulation. This microcracking is seen optically as “whitening” of the bone specimen during yield, long before a fatal crack develops.<sup>67</sup> Staining techniques have been used to examine microcracking in biomineral specimens, which may be useful for examining our specimens.<sup>66</sup> Likewise, the fracture surface of mineralized tissues has provided information on the toughening mechanisms, such as for example, crack bridging, interface debonding, frictional pullout, and secondary microcracking.

Fracture mechanics for bone are difficult to assess due to the high degree of variability of specimen samples, and the anisotropic grain of the tissue. Values of  $K_{Ic}$  ranging from 3.2 to 6.6  $\text{MNm}^{-3/2}$  have been recorded.<sup>62, 63</sup> The fracture toughness ( $K_{Ic}$ ) of our nanocomposites will be determined using fractography. The fracture surfaces of the flexural bars will be examined using optical microscopy, and the size of the surface flaw that initiated failure will be measured.  $K_{Ic}$  can then be determined from:<sup>68, 69</sup>

$$K_{Ic} = \frac{1.1\sigma_f[\pi c]^{3/2}}{\phi}$$

where  $\sigma_f$  is the stress at fracture,  $c$  is either the depth or the half-width of the surface flaw that caused failure, whichever is smaller, and  $\phi$  is an elliptical integral of the second kind. If any plastic deformation occurs, then the fracture toughness is determined from:

$$K_{Ic}^2 = \frac{1.2\pi\sigma_f^2 c}{\phi^2 - \frac{0.212\sigma_f^2}{\sigma_{ys}^2}}$$

where  $\sigma_{ys}$  is the yield strength. Quite often the initiating flaw is approximately semi-spherical, in which case the flaw can be assumed to having a radius  $c=(ab)^{1/2}$ , with  $\phi=1.57$ . These equations are only valid for mode I (tensile) loading. Thus, they can only be used for flexural tests if the failure initiates in the region of tension. Also, the equation for plastic deformation assumes that the radius of the plastic zone is small compared to the crack tip. Thus, it will be important to examine the fracture surfaces carefully to ensure that these assumptions are valid for each specimen tested.

We have chosen this technique for determining fracture toughness over other, more conventional techniques, because both flexural properties and fracture toughness can be determined in a single test. Conventional fracture toughness measurements require preparation of samples designed specifically for those measurements. Since we can utilize the same sample for both measurements, fewer samples are needed and the fracture toughness measurements can be carried out within the scope of this Phase I project.

#### **4. Broad Impact of Proposed Research**

In general terms, Phase I of this project will examine a new processing technique for the development of a unique class of polymer-ceramic composites. The properties of the ceramic phase will be mediated by the organic matrix, similar to what occurs biologically during bone formation. It is anticipated that this will provide a new toughening mechanism to the ceramic phase, or vice versa, it will provide stiffening and strengthening to the polymeric phase. More specifically, this exploratory research will lead to Phase II, which will develop new strategies for designing and fabricating hard-tissue biomaterials. If the materials engineer is able to mimic the biological processes of intrafibrillar mineralization of collagen, then it is anticipated that hard-tissue biomaterials can be fabricated with greatly enhanced mechanical properties, along with the necessary bioresorptive potential for natural tissue regeneration. The PILP process can conceivably generate a heavily-loaded collagen matrix that, due to the high density of mineral phase, will provide compressive strengths capable of load-bearing applications in bone-graft substitutes. The nanostructured architecture of the HAP crystallites within the composite should provide the capability of toughening the ceramic biomaterial in a manner that is achieved biologically, such as through microcracking accumulation. In terms of biocompatibility, because the chemical composition as well as the architecture of the artificial biomaterial will simulate the environment of natural bone tissue, it is anticipated that this biomimetic approach will enable the engineering of biomaterials that are osteoinductive for stimulating natural tissue regeneration. The potential for bioresorptivity of a bone mimic is particularly advantageous in that it could continue to carry a load as it is remodeled under biological control. Additionally, the low-temperature aqueous-based processing conditions of the PILP process can be exploited for the incorporation of biological components, such as bone morphogenetic proteins, and possibly even osteoprogenitor cells. Overall, the broad impact of this research to society is to provide a new synthetic route towards the fabrication of bone-graft substitutes. Autografts (e.g. bone harvested from iliac crest) are the current gold standard for critical sized osseous defects (i.e. greater than 2 cm); but this requires an extra surgery for harvesting the donor bone and commonly leads to donor site morbidity. An alternative is to use allografts (cadaver bone), but this can have problems with disease transmission and infection, and since it does not contain live cells, it is not as osteogenic as autografts. With the more than 426,000 bone-graft procedures performed in the U.S. in 1995,<sup>11</sup> there is a critical need to develop alternative materials for hard-tissue engineering.

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## BIOGRAPHICAL SKETCH

**Dr. Elliot P. Douglas**

### **A. Vita**

#### Address:

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### **Education**

Ph.D., Polymer Science and Engineering, 1993, University of Massachusetts-Amherst  
S.B., Materials Science and Engineering, 1988, Massachusetts Institute of Technology,  
S.B., Humanities and Engineering, 1988, Massachusetts Institute of Technology,

### **Work Experience**

University of Florida, Assistant Professor, Department of Materials Science and Engineering,  
August, 1996 - present  
Los Alamos National Laboratory, Polymer Team Leader, June, 1995 - August, 1996  
Los Alamos National Laboratory, Technical Staff Member, October, 1993 - June, 1995  
Los Alamos National Laboratory, Postdoctoral Associate, September, 1992 - October, 1993

### **Recent Awards**

Presidential Early Career Award for Scientists and Engineers, 1997  
Los Alamos National Laboratory Excellence in Industrial Partnerships Award, 1996

### **B. Publications**

#### Related to proposal:

1. Elliot P. Douglas, "Magnetic field orientation of liquid crystalline thermosets: Orientation kinetics", *Polymer Preprints*, **1999**, 40 (2), 498-499
2. Derek M. Lincoln and Elliot P. Douglas, "Control of orientation in liquid crystalline epoxies via magnetic field processing", *Polymer Engineering and Science*, in press
3. Brian C. Benicewicz, Mark E. Smith, Jim D. Earls, Ralph D. Priester, Jr., Stefan M. Setz, Randolph S. Duran, and Elliot P. Douglas, "Magnetic field orientation of liquid crystalline epoxy thermosets", *Macromolecules*, **1998**, 31, 4730
4. Brian C. Benicewicz, Mark E. Smith, Jim D. Earls, Ralph D. Priester, Jr., and Elliot P. Douglas, "Novel routes to high strength, light weight materials: Magnetic field processing of liquid crystalline thermosets", *ChemTech*, **1997**, 27(8), 44

5. Elliot P. Douglas, Mark E. Smith, Brian C. Benicewicz, Jim D. Earls, and Ralph D. Priester, Jr., "Processing of polymers in high magnetic fields", in High Magnetic Fields: Applications, Generation, Materials, Hans J. Schneider-Muntau, ed., World Scientific Publishing Co., River Edge, NJ, 1997, p. 31

Other significant publications:

6. Elliot P. Douglas, Arthur J. Gavrin, and Tonya Bervaldi, "Isothermal degradation of a novel phenylethynyl liquid crystalline thermoset", *Polymer Preprints*, **1999**, 40 (2), 516-517
7. Arthur J. Gavrin, Christine L. Curts, and Elliot P. Douglas, "High temperature stability of a novel phenylethynyl liquid crystalline thermoset", *Journal of Polymer Science: Part A: Polymer Chemistry*, in press
8. David A. Langlois, Brian C. Benicewicz, and Elliot P. Douglas, "Liquid crystalline bispropargyl thermosets", *Chemistry of Materials*, **1998**, 10, 3393
9. Rex P. Hjelm, Elliot P. Douglas, and Brian C. Benicewicz, "The solution structure of liquid crystal polymers with small liquid crystal thermosets", *International Journal of Thermophysics*, **1995**, 16, 309
10. Elliot P. Douglas, David A. Langlois, and Brian C. Benicewicz, "Synthesis, phase behavior, and curing studies of bisacetylene rigid rod thermosets", *Chemistry of Materials*, **1994**, 6, 1925

**C. Other Collaborators**

Dominick Cangiano, Hoechst-Celanese Corporation  
Jim Earls, The Dow Chemical Company  
Rex Hjelm, Los Alamos National Laboratory  
David Langlois, Los Alamos National Laboratory  
Ralph Priester, The Dow Chemical Company  
Mark Smith, Los Alamos National Laboratory  
P. Thiyagarajan, Argonne National Laboratory  
Ed Wilburn, Wellman, Inc.

**D. Students**

Derek M. Lincoln, Wright-Patterson AFB  
Total of 1 graduate student, 0 postdoctoral scholars advised.

**E. Graduate and Postdoctoral Advisors**

graduate advisor: Dr. William J. MacKnight, University of Massachusetts  
postdoctoral advisor: Dr. Brian C. Benicewicz, Los Alamos National Laboratory (currently at RPI)

## **E. BIOGRAPHICAL SKETCH**

### **a. Vita**

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### **Educational Background**

1997 Ph.D. Polymer Science & Engineering, University of Massachusetts, Amherst, MA  
1992 M.S. Bioengineering, University of Utah, Salt Lake City, UT  
1985 B.S. Engineering Science, University of Florida, Gainesville, FL

### **Professional Experience**

1997 - present Asst. Professor, University of Florida, Dept. of Materials Science & Engineering  
1997 - 1998 Consultant, Specialty Minerals Inc., Bethlehem, PA  
1991 - 1997 Graduate Research Assistant, University of Massachusetts at Amherst, MA  
1988 - 1990 Graduate Research and Teaching Assistant, University of Utah, UT  
1985 - 1987 Research Assistant, UCAR Emulsion Systems, Cary, NC

### **Research Interests**

Biomimetics, biomineralization, polymeric crystal growth modifiers, engineered particulates, ceramic/polymer composites, ceramic thin films, nano-, meso- and hierarchical structures, biomedical materials, tissue engineering.

### **Courses Taught**

EMA 6938: Graduate special topics course on "Biomimetics & Biomineralization" (Spring '98)  
EMA 3010: Undergraduate course on "Introduction to Materials" (Fall '98, Spring '99)  
EMA 3066: Undergraduate course on "Introduction to Polymers" (scheduled for Fall '99)

### **b. Publications**

1. Gower, L.A., T.-L.D. Wang, and D.J. Lyman, "Morphology of Block Copolyurethanes: V. The Effect of -CH<sub>2</sub>CH<sub>2</sub>- versus -CH<sub>2</sub>- Spacers Between Aromatic Rings," *Journal of Biomaterials Science., Polymer Edition*, **6**, 8, 761-73 (1994).
2. Gower, L.A. and D.J. Lyman, "Phase Separation of Copolyurethanes: A Study of Annealing and Slow Solvent Evaporation Methods by FTIR," *Journal of Polymer Science, Part A: Polymer Chemistry*, **33**, 13, 2257-66 (1995).

3. Lyman, D.J., and L.A. Gower, "Effect of Infrared Salt Crystals on the Spectra of Copolyether-urethane-urea Films," *Vibrational Spectroscopy*, **9**, 2, 203-7 (1995).
4. Gower, L.A., T.-L.D. Wang, and D.J. Lyman, "Morphology of Block Copolyurethanes: VI. The Effect of Segmental Dispersity on Phase Separation," in preparation.
5. Gower, L.A., "The Influence of Polyaspartate Additive on the Growth and Morphology of Calcium Carbonate Crystals," Ph.D. Dissertation, University of Massachusetts (1997).
6. Gower, L.A. and D.A. Tirrell, "Calcium Carbonate Films and Helices Grown in Solutions of Polyaspartate," *Journal of Crystal Growth*, 191,1-2, (1998).
7. Gower, L.A. and D.A. Tirrell, "Polymer Induced Deposition of Calcium Carbonate Films Via a Liquid Precursor," *Journal of Crystal Growth*, in revision (1999).
8. Gower, L.A. and E.A. CoBabe, "Relevance of a Polymer-Induced Liquid-Precursor Process to Calcium Carbonate Biomineralization," in preparation.

**c. Collaborators (proposals have been submitted with the following faculty at UF)**

Kenneth Anusavice; Dept. of Dental Biomaterials  
 Ron Baney; Department of Materials Science & Engineering  
 Richard Dickinson; Department of Chemical Engineering  
 Elliot Douglas; Department of Materials Science & Engineering  
 Randy Duran; Department of Chemistry  
 Hassan El-Shall; Department of Materials Science & Engineering  
 Guenther Hochhaus; Department of Pharmaceutics  
 Saeed Khan; Dept. of Pathology  
 Jack Mecholsky; Dept. of Materials Science & Engineering  
 Brij Moudgil; Department of Materials Science & Engineering  
 Dinesh Shah; Department of Chemical Engineering  
 Daniel Talham; Dept. of Chemistry  
 Donna Wheeler; Dept. of Orthopaedics  
 Wolfgang Sigmund; Department of Materials Science & Engineering

**d. Students Advised**

Within the first two years of appointment, the PI has advised 6 graduate students (one as co-advisor), 4 undergraduate students on senior research projects, and one summer REU student.

**e. Former Graduate Advisors**

Doctoral Work: Dr. David A. Tirrell, Barrett Professor,  
 University of Massachusetts at Amherst  
 (currently Prof. at California Institute of Technology)

Master's Work: Dr. Donald J. Lyman, Professor Emeritus  
 University of Utah (currently residing in Seattle Washington)

## **H. FACILITIES, EQUIPMENT AND OTHER RESOURCES**

**Laboratory:** Dr. Douglas's laboratory is equipped with mechanical testing facilities and an optical microscope for use on this project, as well as standard hoods and ovens. Dr. Gower's laboratory is equipped with instruments and supplies for crystallization assays, as well as hoods and ovens for sample preparation. A polarizing microscope with long-working-distance objectives can be used to monitor crystallizations in situ, and the microscope is equipped with a color 3-chip CCD camera, framegrabber and computer for particle analysis. The microscope also has fluorescence capability for monitoring labeled additives.

**Computer:** The student and PI offices are equipped with computers, as well as a high-memory computer in the microscopy laboratory equipped with a framegrabber for digital image analysis. We are currently using the downloadable NIH Image software for image analysis.

**Office:** Office area for both the PI and students include needed office supplies and computers, with network connections and up to date Office, data processing, and Graphics software. The offices are conveniently located next to the laboratory.

**Clinical and Animal:** N.A. for this project. In future work dealing with biomaterials applications, experimental facilities for animal testing and clinical tests are available through collaboration with faculty in the Colleges of Medicine or Dentistry (Shands Teaching Hospital).

**Other:** Magnetic field processing will be conducted at the National High Magnetic Field Laboratory, Tallahassee, FL. The NHMFL is a world-class magnetic science facility. The PI has conducted many experiments at the NHMFL.

A beamline at the Advanced Photon Source at Argonne National Laboratory is available for use through the membership of the University of Florida in the Materials Research Collaborative Access Team (MR-CAT), directed by Dr. Randy Duran from the Department of Chemistry. The synchrotron facilities may be useful in this investigation for examining the crystalline orientation relative to templates, and texture of the mineral phase.

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### **MAJOR EQUIPMENT:**

The Major Analytical Instrumentation Center (MAIC), conveniently located within the PI's home department, is equipped with large analytical instruments that cannot be maintained by a single investigator. The center includes a 200 kV and 400 kV TEM, which can be used for both direct imaging and electron diffraction, HR-TEM; several SEMs, and a digital scanning probe microscope (ambient STM/AFM); and x-ray diffractometers, including a HR-XRD.

A user fee is charged on a per hour basis for use of instruments in the MAIC facility.

The Department of Chemistry maintains a comprehensive NMR facilities, which also charges a users fee. The NSF Engineering Research Center (ERC) on Particle Science & Technology has extensive facilities for particle analysis and characterization, including zeta potentiometer, ICP, light scattering, and microscopic FTIR. In addition, the ERC/MSE Department has recently received a grant to purchase a Scanning Transmission Electron Microscope with atomic number (Z) contrast (STEM-Z) imaging. Gower is an faculty member of the ERC, and therefore a user fee is not charged for ERC instrumentation.